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14. ABSTRACT
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a. REPORT	b. ABSTRACT	c. THIS PAGE			Steven Benner
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Report Title

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Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received	Paper

TOTAL:

Number of Manuscripts:

Books

Received	Paper

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Nicole Leal	1.00
Hyo Joong Kim	1.00
FTE Equivalent:	**2.00**
Total Number:	**2**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Under Graduate students supported

NAME	PERCENT SUPPORTED

FTE Equivalent:

Total Number:

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	0.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:.....	0.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:.....	0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	0.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME	PERCENT SUPPORTED

FTE Equivalent:

Total Number:

Sub Contractors (DD882)

1 a. Foundation for Applied Molecular Evolution

1 b. 1115 NW 45th Street

Gainesville FL 32601

Sub Contractor Numbers (c):

Patent Clause Number (d-1):

Patent Date (d-2):

Work Description (e):

Sub Contract Award Date (f-1): 6/1/2011 12:00:00AM

Sub Contract Est Completion Date(f-2): 4/30/2012 12:00:00AM

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1 a. Foundation for Applied Molecular Evolution

1 b. PO Box 13174

Gainesville FL 32604

Sub Contractor Numbers (c):

Patent Clause Number (d-1):

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Sub Contract Award Date (f-1): 6/1/2011 12:00:00AM

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Inventions (DD882)

Scientific Progress

Technology Transfer

Design Automation Software for DNA-based Nano-Sensor Architecture

W911NF-11-C-0086

Report

Principal Investigator: Steven A. Benner
Date: April 30, 2012
Organization: Firebird Biomolecular Sciences LLC

Rationale

This project was developed in response to an RFA seeking innovations to create DNA molecules that self-assemble to support nanomolecular design. While the solicitation emphasized software to solve the challenges arising from the intrinsic chemistry of DNA, which is built from just four nucleotides (GACT), the premise of this project was that the design task could be rendered trivial if standard DNA were augmented extra nucleotides to give an "artificially expanded genetic information system" (AEGIS). These are shown in Figure 1.

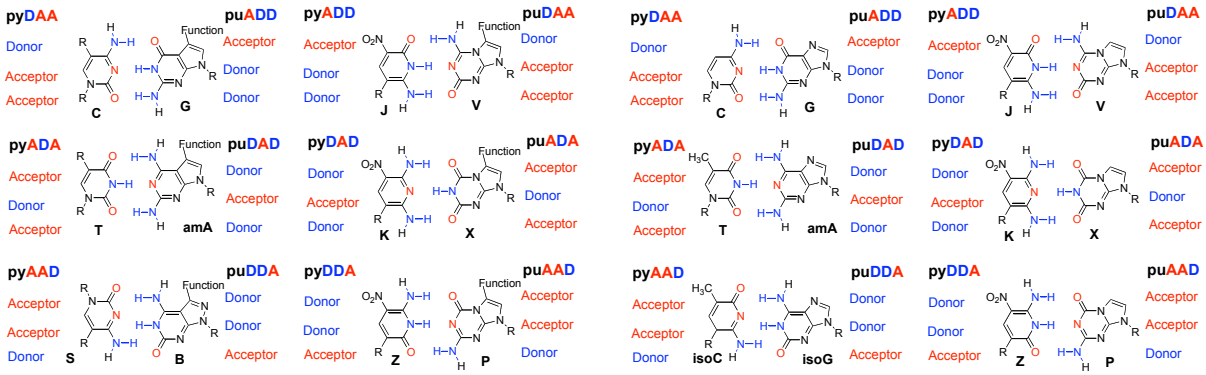

Figure 1. The AEGIS components optimally designed to support nanostructure design (left); compare with the standard versions of the AEGIS components (right). R and "Function" indicate sites for potential functionalization of these structures.

Design of experiments to collect data to support software development

In anticipation of Phase 2, we have turned to experiments that need to be done to support key customers who will wish to use AEGIS DNA. This month we have focused on those who will want to use “ultra long” DNA (UL-DNA) constructs. This has focused on developing a bacterial strain that will be able to manage plasmids containing AEGIS DNA.

To this end, we asked what happens to Z/P containing DNA when introduced into *E. coli*. Here, plasmids containing an insert with targeted Z:P pairs were used to transform DH5α and BL21-gold host bacteria (the strains are rec A1 recombination deficient, not SOS deficient; and recA+, respectively).

Surprisingly, having Z/P in the plasmid did not lower the transformation efficiency of the construct when compared to a control with an insert lacking Z:P base pairs. Fifty clones were screened for presence of the insert by PCR; only three did not have the insert. This means that the clones had not excised the insert and reformed the plasmid, which generally happens when an insert is toxic. Since these cells were not supplied with dZ:dP nucleoside or the Dm-dNK kinase they must have substituted natural nucleosides for them. Further, since the colony counts and growth rate between control insert and Z:P containing insert were the same, replacement of the Z:P pairs seems to happen quickly.

Sequencing of the insert contained in plasmids purified from these clones showed an interesting pattern of substitution (Table 1). All ZZ:PP dinucleotides were converted to CC:GG dinucleotides. This will be helpful when designing selections to create UL-DNA molecules.

As another rule, P was observed to always beconverted to G or A, while Z is always converted to C or T. The rule suggests that the polymerase is matching size

Table 1. Sequencing of converted P:Z inserts from DH5α and BL21-gold

Data Set	Target Sequence	# of sequences	P:Z to G:C	P:Z to A:T	Z:P to C:G	Z:P to T:A	% converted to G:C	% converted to C:G
1Z	APg/TZC	24	15	9			62.5	
2Z	APPg/TZZC	29	29	0			100	
2ZP	gPggCCZC/							
	CZCCggPg	30	23	7	16	14	77	53

These results, showing predicable conversion of Z:P pairs, will be a key technology to support customers wishing to use the orthogonality of Z:P pairs to construct UL-DNA.